Endogenous estrogens increase postischemic hyperemia in the skin microcirculation

Noll, G ; et al ; Lüscher, T F

DOI: https://doi.org/10.1097/01.fjc.0000157457.83809.94

Endogenous Estrogens Increase Postischemic Hyperemia in the Skin Microcirculation

Vesna Stojanovic, Fides Küng,† Lukas E. Spieker,* Christian Binggeli,* Isabella Sudano,* Daniel Hayoz,‡ Thomas F. Lüscher,* and Georg Noll**

Abstract: Estrogens have been recognized as a major regulator of vascular tone and structure, particularly in the skin. The objective of this study was to investigate the effects of endogenous estrogens on the skin microcirculation. Skin blood flow was measured at the forearm at rest and during postischemic hyperemia using laser Doppler flowmetry in 32 healthy women (mean age 34.5 ± 3.9 years) involved in an in-vitro fertilization program. Women were treated for 10 to 12 days with gonadotropin-releasing hormone agonist (total dose 40.3 ± 3.3 mg) and human menopausal gonadotropin (1942 ± 801 IE) or follicle-stimulating hormone (2544 ± 1071 IE) according to individual estrogen levels. Plasma estrogen levels increased from 132 ± 90 pmol/L (36 ± 25 pg/mL) to 8471 ± 4386 pmol/L (2308 ± 1195 pg/mL) during treatment ($P < 0.0001$). Maximal hyperemic blood flow increased from 353 ± 81% before treatment to 516 ± 144% after hormonal stimulation ($P < 0.0001$), whereas basal skin flow was not altered. This study shows that endogenous estrogens enhance the postischemic hyperemic response of the skin microcirculation.

Key Words: estrogen, skin microcirculation, hyperemia

(*J Cardiovasc Pharmacol*™ 2005;45:414–417)

Estrogens exert profound and broad actions on the vasculature. Indeed, clinical observation of the skin before and after menopause as well as during pregnancy suggest an important role of female sex hormones for skin perfusion and metabolism. In the coronary circulation, estrogen acts as a vasodilator.[1] Estrogens modulate the production of endothelium-derived vasoactive substances, eg, nitric oxide (NO)[2] and endothelin-1 (ET-1), and thus influence vascular tone and structure.[3] Estrogens also influence the actions of ET-1 at the ET_B receptor level.[4] NO causes vasodilatation, inhibits platelet aggregation, suppresses smooth-muscle cell proliferation, and acts as an antiatherogenic factor.[5] ET-1 opposes the effects of NO, causes potent vasoconstriction of the systemic and pulmonary vasculature, promotes monocyte adhesion to the vascular wall, activates macrophages, and promotes vascular smooth-muscle cell proliferation. Continuous release of NO and ET-1 from the endothelium influences basal vascular tone.[6,7]

We have previously demonstrated that postischemic hyperemia in the skin microcirculation is dependent on vasodilator prostaglandins.[8] The precise effects of endogenous estrogens on the microcirculation still remain elusive. The aim of this study, therefore, was to investigate the impact of endogenous estrogens on the skin microcirculation during stimulation of endogenous estrogen production in healthy women.

MATERIALS AND METHODS

Study Population

Thirty-two women with an average age of 34 ± 3.9 years were studied during an in-vitro fertilization program. To be considered eligible for the study women had to be in reproductive period, with regular menstrual cycles of between 26 and 32 days during the last 6 months. Exclusion criteria were clinical or laboratory evidence of endocrine disease; a history of renal, respiratory, hematological, neurologic, or gastrointestinal disease; clinically significant ophthalmic abnormalities; malignancy; or chronic alcohol or drug abuse. As control group, 7 women not showing the expected increase in estrogen levels were studied. These women were considered nonresponders to stimulation therapy as they were not showing the expected >50-fold increase in estradiol levels. The control group was studied in parallel fashion under the same conditions to account for possible confounding factors.

Protocol

Women were treated for 10 to 12 days with gonadotropin-releasing hormone-agonist (GnRHa) with an average total dose of 40.3 ± 3.3 mg. They were additionally treated with human menopausal gonadotropin (HMG, average total dose 1942 ± 801 IE), or follicle stimulating hormone (FSH, average total dose 2544 ± 1071 IE). The dosing of FSH/HMG was chosen according to individual estrogen levels.

Measurement of Skin Reactive Hyperemia

A laser Doppler flowmeter (PF3, Perimed, Sweden) with a probe holder was used to assess skin blood flow as previously described.[8] The laser probe was placed on the forearm distal from a blood pressure cuff loosely placed around the upper

Received for publication September 4, 2004; accepted January 13, 2005.

From the *Cardiovascular Center, Department of Cardiology, University Hospital, Zürich, Switzerland; †Department of Obstetrics and Gynecology, University Hospital, Zürich, Switzerland; and ‡Division of Hypertension and Vascular Medicine, Centre Hospitalier Universitaire, Vaudois, Lausanne, Switzerland.

This work was supported by the Swiss National Research Foundation (grants 32-51069.97/1 and 32-52690.97), the Swiss Heart Foundation, and the Stanley Thomas Johnson Foundation.

Reprints: Georg Noll, MD, FESCCardiology, University Hospital, Raemistrasse 100, CH-8091 Zürich, Switzerland (e-mail: karnog@usz.unizh.ch).

arm. Stable baseline conditions were recorded for 2 minutes. Then, the cuff was inflated to suprasystolic pressure for 4.5 minutes. After release of the cuff, skin reactive hyperemia was recorded. The measurement of skin blood flow is different from techniques for assessment of whole forearm blood flow such as venous occlusion plethysmography in that it specifically measures skin vessels. In contrast to ultrasonography of large arteries, conduit vessel function is not assessed by laser Doppler flowmetry of the skin.

All patients were studied under standardized conditions, ie, in the morning (8:00 AM) in a temperature-controlled room (23°C). Subjects abstained from smoking for 24 hours, from caffeine- and alcohol-containing beverages for 12 hours, and from food for 2 hours before the test. Blood pressure was measured with appropriate cuff size after the patient had been supine for at least 15 minutes.

Biochemical Analyses

Blood samples were taken before treatment and after 5, 8, and 10 to 12 days of treatment. Serum levels of 17β-estradiol and progesterone were measured by radioimmunoassay. Levels are reported as picomoles/liter as well as picograms/milliliter (to convert, divide by 3.671).

Statistical Analysis

Basal skin blood flow, peak skin blood flow, and half-life of the hyperemic response were calculated (Matlab, Math Works, Natick, MA). Results represent arbitrary flow units. Maximal blood flow was normalized to resting blood flow and expressed as percentage increase.

Values represent means ± SEM. Single comparisons were made with paired and unpaired Student *t* test. Effects of treatment were compared using ANOVA for repeated measurements (StatView 4.5, Abacus Concepts, Berkeley, CA). A *P* value of 0.05 or less was considered statistically significant.

RESULTS

The clinical characteristics of the study participants are shown in Table 1. During the in-vitro fertilization program, 17β-estradiol plasma levels significantly increased (Table 1). There was an accompanying significant increase in blood pressure and body weight.

Basal skin blood flow did not change during the study course (Table 2). Hyperemic blood flow after cuff deflation increased from 353 ± 81% to 516 ± 144% of baseline blood flow ($P < 0.0001$, Table 2 and Fig. 1). Body temperature was comparable before and after therapy (36.5 ± 0.4 and 36.8 ± 0.6°C, ns).

In the control group consisting of women only poorly responding to stimulation with an increase in 17β-estradiol levels (from 75 ± 63 to 967 ± 823 pmol/L, $P = 0.0007$; ie, 20 ± 17 to 263 ± 224 pg/mL), the increase in maximal postischemic blood flow was blunted (from 411 ± 155% to 438 ± 171%, $P = 0.068$).

TABLE 1. Clinical Parameters During the Study Course

	17ß-estradiol (pmol/L)	SBP (mm Hg)	DBP (mm Hg)	HR (bpm)	BMI (kg/m^2)
Day 1	132 ± 90	109 ± 11	72 ± 6	76 ± 11.6	24.8 ± 3.5
Day 5	2290 ± 1002	112 ± 9	73 ± 7	76 ± 10.5	25.5 ± 3.6
Day 8	5144 ± 2365	118 ± 11	75 ± 6	76 ± 11.6	25.6 ± 3.6
Day 10	8471 ± 4386*	123 ± 9*	78 ± 5†	78 ± 10.0	25.8 ± 3.5*

DPB denotes diastolic blood preassure; SBP, systolic blood pressure; HR, heart rate; BMI, body mass index. To convert estradiol levels from pmol/L to pg/mL, divide by 3.671.

*$P \leq 0.0001$, †$P = 0.0024$ by ANOVA.

TABLE 2. Changes in Skin Blood Flow During Estrogen-Stimulating Therapy

	Basal Skin Blood Flow (Units)	Maximal Postischemic Blood Flow (Units)	Half-Life of Hyperemic Response (Seconds)
Day 1	15.6 ± 4.8	65 ± 20	43 ± 18
Day 5	14.6 ± 4.0	73 ± 24	39 ± 17
Day 8	14.7 ± 5.1	80 ± 28	39 ± 13
Day 10	13.7 ± 3.2	88 ± 27*	32 ± 11

*$P < 0.0001$ by ANOVA.

DISCUSSION

The present study demonstrates for the first time that endogenous estrogens have a profound influence on the regulation of blood flow in the skin microcirculation. The increase in estrogen levels after hormone therapy was associated with enhanced postischemic skin blood flow, although there was no change in basal skin perfusion.

Reactive hyperemia of the skin bases is determined by a complex interplay of several vasoactive substances including NO, prostaglandins, adenosine, ATP-sensitive potassium channels, and oxygen. We have previously demonstrated that postischemic hyperemia of the skin is mainly dependent on vasodilator prostaglandins.[8] This is in contrast to large peripheral conduit arteries where nitric oxide (NO) is mainly

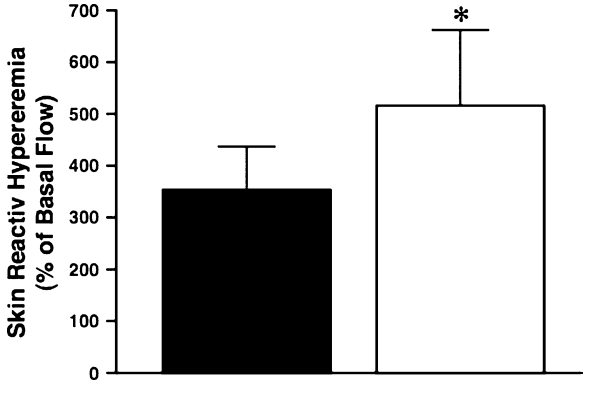

FIGURE 1. Reactive hyperemia in the skin before and after 10 days of estrogen stimulation in healthy women. Stimulation of endogenous estrogen release led to an increase in postischemic reactive hyperemia of the skin (*$P < 0.0001$). Basal skin blood flow did not change during the study course.

responsible for flow-mediated vasodilation.[9] Postischemic skin hyperemia is impaired in hypercholesterolemic patients and can be enhanced by cholesterol-lowering therapy.[8,10] Because laser Doppler flowmetry is a simple noninvasive method for measuring skin blood flow during reactive hyperemia, it can be used clinically to test and monitor vascular function during physiological or therapeutic interventions.[8]

The present study shows that in healthy women of gestational age without cardiovascular risk factors, estrogens have profound impact on microvascular function. Postischemic skin reactive hyperemia considerably increased in these women participating in an in vitro fertilization program resulting in pronounced estrogen stimulation (about 60-fold increase in estradiol levels). In the control group consisting of women who showed only a moderate increase in estrogen levels (about 12-fold), the increase in postischemic blood flow was blunted. In untreated women not undergoing estrogen stimulation, estradiol levels typically increase 10-fold at the time of ovulation with large interindividual variation.

Estrogens exert both rapid and long-term effects on the endothelium and are involved in the flow-mediated and shear stress–induced release of vasoactive substances.[3] On the basis of our and others' previous work investigating the mechanisms involved in postischemic reactive hyperemia of the skin,[8,11] an enhancement of vasodilator prostaglandins may be responsible for the improvement of reactive hyperemia induced by increased estrogen levels in the present study. These mechanisms are different from those in flow-induced dilatation of large peripheral and coronary arteries.[9] However, impaired endothelial function in postmenopausal women is improved by hormone replacement.[12]

Interestingly, basal skin perfusion was not altered by estrogen stimulation, although alterations in skin perfusion occur during a normal menstrual cycle.[13,14] Basal skin perfusion is largely influenced by NO.[15] These findings delineate the differences in the regulation of the skin and muscle microcirculation. NO is an important regulator of basal blood flow in the skeletal and myocardial microcirculation. Because hormone replacement therapy does not affect basal or stimulated skin blood flow in postmenopausal women,[16] the question arises whether endogenous and exogenous estrogens have profoundly different effects on the circulatory regulation. The disappointing results from hormone replacement therapy trials in postmenopausal women with coronary artery disease support this view. Although myocardial ischemia in women with stable angina is reduced by hormone replacement therapy,[17] large clinical trials yielded no benefit in terms of mortality.

Besides of the role of estrogens in the normal aging skin, the regulation of skin perfusion is of interest in many clinical conditions. Raynaud phenomenon causes significant symptoms in affected patients. Female patients with Raynaud phenomenon show altered regulation of skin perfusion during the menstrual cycle.[18] As stimulation of endogenous estrogens leads to a potentiation of postischemic skin blood flow, the present results are in line with a major role of estrogens in the regulation of skin perfusion and thermoregulation.[19–21] Such an important regulator function helps to explain alterations in thermoregulation of the skin in postmenopausal versus premenopausal women, which is often responsive to estrogen replacement therapy.[21–24] Moreover, the postischemic response to reperfusion injury depends on estrogen status.[25] Delayed wound healing in the elderly also responds to topical estrogen administration,[26] as inflammatory mechanisms provoke hyperemia of the skin, which may be increased by estrogens.

In addition to the observed alterations in skin perfusion, the increase in 17β-estradiol levels was accompanied by an increase in systolic and diastolic blood pressure. Also, there was an increase in body weight. The observed hemodynamic changes are thus likely caused by estrogen-induced volume retention. Activation of the renin–angiotensin system may have contributed.

The present study is limited by the fact that it is not randomized and placebo-controlled. However, the observed differences were indeed large; thus, minor and accidental variations on a daily basis are unlikely to play a role. Another limiting factor is the regulation of skin blood flow by other systems than the endothelium, eg, the sympathetic nervous system. Skin temperature might influence postischemic skin blood flow. However, this was monitored closely in our study and cannot explain the improvement of reactive hyperemia. Performing the experiments in an air-conditioned and quiet room minimized these confounding influences, eg, noise and changing temperature, as well.

In conclusion, this study demonstrates that endogenous estrogens improve postischemic blood flow in the skin microcirculation. These results underline the regulatory role of endogenous estrogens in the normal aging skin and in many clinical conditions with dysregulated cutaneous perfusion.

REFERENCES

1. Hutchison SJ, Chou TM, Chatterjee K, et al. Tamoxifen is an acute, estrogen-like, coronary vasodilator of porcine coronary arteries in vitro. *J Cardiovasc Pharmacol*. 2001;38:657–665.
2. Yang S, Bae L, Zhang L. Estrogen increases eNOS and NO_x release in human coronary artery endothelium. *J Cardiovasc Pharmacol*. 2000;36: 242–247.
3. Mendelsohn ME, Karas RH. Mechanisms of disease: The protective effects of estrogens on the cardiovascular system. *N Engl J Med*. 1999; 340:1801–1811.
4. Barber DA, Sieck GC, Fitzpatrick LA, et al. Endothelin receptors are modulated in association with endogenous fluctuations in estrogen. *Am J Physiol*. 1996;271:H1999–H2006.
5. Spieker LE, Luscher TF, Noll G. Current strategies and perspectives for correcting endothelial dysfunction in atherosclerosis. *J Cardiovasc Pharmacol*. 2001;38(Suppl 2):S35–S41.
6. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide onperipheral arteriolar tone in man. *Lancet*. 1989;2:997–1000.
7. Haynes WG, Webb DJ. Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet*. 1994;344:852–854.
8. Binggeli C, Spieker LE, Corti R, et al. Statins enhance post-ischemic hyperemia in the skin circulation of hypercholesterolemic patients: A monitoring test of endothelial dysfunction for clinical practice? *J Am Coll Cardiol*. 2003;41:71–77.
9. Joannides R, Haefeli WE, Linder L, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*. 1995;91:1314–1319.
10. Fagrell B. Dynamics of skin microcirculation in humans. *J Cardiovasc Pharmacol*. 1985;7(Suppl 3):S53–S58.
11. Calkin AC, Sudhir K, Honisett S, et al. Rapid potentiation of endothelium-dependent vasodilation by estradiol in postmenopausal women is mediated via cyclooxygenase 2. *J Clin Endocrinol Metab*. 2002;87: 5072–5075.

12. Tagawa H, Shimokawa H, Tagawa T, et al. Short-term estrogen augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilation in postmenopausal women. *J Cardiovasc Pharmacol*. 1997;30:481–488.
13. Bartelink ML, Wollersheim H, Theeuwes A, et al. Changes in skin blood flow during the menstrual cycle: the influence of the menstrual cycle on the peripheral circulation in healthy female volunteers. *Clin Sci (Lond)*. 1990;78:527–532.
14. Bungum L, Kvernebo K, Oian P, et al. Laser Doppler-recorded reactive hyperaemia in the forearm skin during the menstrual cycle. *Br J Obstet Gynaecol*. 1996;103:70–75.
15. Charkoudian N, Stephens DP, Pirkle KC, et al. Influence of female reproductive hormones on local thermal control of skin blood flow. *J Appl Physiol*. 1999;87:1719–1723.
16. Brooks-Asplund EM, Kenney WL. Chronic hormone replacement therapy does not alter resting or maximal skin blood flow. *J Appl Physiol*. 1998;85: 505–510.
17. Sanderson JE, Haines CJ, Yeung L, et al. Anti-ischemic action of estrogen-progestogen continuous combined hormone replacement therapy in postmenopausal women with established angina pectoris: a randomized, placebo-controlled, double-blind, parallel-group trial. *J Cardiovasc Pharmacol*. 2001;38:372–383.
18. Greenstein D, Jeffcote N, Ilsley D, et al. The menstrual cycle and Raynaud's phenomenon. *Angiology.* 1996;47:427–436.
19. Pang CY, Chiu C, Zhong A, et al. Pharmacologic intervention of skin vasospasm and ischemic necrosis in pigs. *J Cardiovasc Pharmacol*. 1993; 21:163–171.
20. Khan F, Palacino JJ, Coffman JD, et al. Chronic inhibition of nitric oxide production augments skin vasoconstriction in the rabbit ear. *J Cardiovasc Pharmacol*. 1993;22:280–286.
21. Charkoudian N, Johnson JM. Reflex control of cutaneous vasoconstrictor system is reset by exogenous female reproductive hormones. *J Appl Physiol*. 1999;87:381–385.
22. Brooks EM, Morgan AL, Pierzga JM, et al. Chronic hormone replacement therapy alters thermoregulatory and vasomotor function in postmenopausal women. *J Appl Physiol*. 1997;83:477–484.
23. Chang RT, Lambert GP, Moseley PL, et al. Effect of estrogen supplementation on exercise thermoregulation in premenopausal women. *J Appl Physiol*. 1998;85:2082–2088.
24. Dunbar SL, Kenney WL. Effects of hormone replacement therapy on hemodynamic responses of postmenopausal women to passive heating. *J Appl Physiol*. 2000;89:97–103.
25. Beer S, Reincke M, Kral M, et al. Susceptibility to cardiac ischemia/reperfusion injury is modulated by chronic estrogen status. *J Cardiovasc Pharmacol*. 2002;40:420–428.
26. Ashcroft GS, Greenwell-Wild T, Horan MA, et al. Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. *Am J Pathol*. 1999;155:1137–1146.

